

REVIEW ARTICLE

Glutathione S-Transferase: An Overview on Distribution of *GSTM1* and *GSTT1* Polymorphisms in Malaysian and Other Populations

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ABSTRACT

Glutathione s-transferases (GSTs) are the vital enzymes involved in Phase II metabolism to detoxify a wide range of carcinogenic metabolites in the body. GST class mu-1 (*GSTM1*) and GST class theta-1 (*GSTT1*) are the genes encoding for the GST isoenzymes. Nevertheless, both genes were frequently reported absent (null) in most of the populations at different frequencies. Null polymorphism will affect the production of GSTs and impair the ability to eliminate carcinogenic compounds which had been shown to expose null individuals to high risk of several cancers such as gastric and lung cancer. Thus, this review will briefly summarize on the *GSTM1* and *GSTT1* polymorphisms, frequencies of null variants in populations worldwide, including Malaysian, and their relevancy to the underlying basis of toxicological response to xenobiotics. Additionally, the genotyping assays used in GST studies will also be discussed.

Keywords: *GSTM1*, *GSTT1*, Genetic polymorphism, Frequency, Genotyping assays

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INTRODUCTION

Phase II metabolism is part of the sequential phases in drug metabolism system, providing the cellular defense against genotoxic molecules introduced into the body (1). Amongst all enzymatic systems constructing the metabolic pathway, Glutathione-s-transferase (GSTs) are the main powerhouse to perform biotransformation or detoxification of harmful substances and to reduce the genotoxicity in the body (2). Low substrate specificity of the GST binding sites also enables the enzyme to tackle a wide range of compounds that can be divided into two types: endobiotics (natively present in human body) and xenobiotics (present in the environment) (2).

GST-encoding genes, GST mu class 1 (*GSTM1*) and GST theta class 1 (*GSTT1*) have been frequently observed absent in most of the human populations. *GSTM1* absents in approximately 50% of Caucasian and Asian populations, and 30% in African descents (3). In contrast, the prevalence of *GSTT1* deletion

is substantially higher in Asians (41%) and lower in Caucasians and Africans with only 20% (3). Thus, these have gradually placed GSTs in a broader scope as the null genotype has been widely reported across various populations, suggesting the genetic polymorphisms are dependent on geographical and ethnic/population factors (2). Loss of the gene functions will alter the expression and functionality of the enzymes and depicts the inter-individual variability in the detoxification system (4). Genetic polymorphism also triggers the onset of various diseases including carcinogenic diseases such as colorectal, prostate, lung, and bladder cancer under the exposure of highly reactive compounds (5–8).

Conventional polymerase chain reaction (PCR) method such as multiplex PCR is commonly used to genotype both genes simultaneously (9). However, the method is only limited to determination of homozygous null genotype and unable to discriminate heterozygosity which can be performed by advanced and quantitative assays such as combination long-range PCR and real-time PCR (10,11). Therefore, the recent state of knowledge encompassing the prevalence of both *GSTM1* and *GSTT1* polymorphism among various populations includes Malaysian, consequences of the polymorphisms toward risk of diseases and appropriate genotyping assays are discussed in detail here.

OVERVIEW

Glutathione s-transferases: Definition and multifunctionality

In xenobiotic or drug metabolic pathway, GSTs are the key player in the detoxification of both xenobiotics and endogenous reactive products of cellular metabolism. These enzymes are responsible to conjugate the hydrophobic reactive compounds to reduced glutathione (GSH) (12). GSTs promote a nucleophilic attack of sulphur atom of the tripeptide GSH on the electrophilic group of the compounds to form less reactive and hydrophilic products. The products are later readily to be eliminated by Phase 3 enzymes (12,13).

Apart from its role in detoxification, GSTs are able to protect body tissue from oxidative damages. GSTs also serve as peroxidases which conjugate GSH to the endogenous products of lipid peroxidation such as epoxides, alkenes and aldehydes (14). In addition, cytosolic GSTs exhibit the properties of isomerase activity that aids the conversion of metabolites or compounds to a more stable form such as the production of prostaglandins (PGD₂, PGE₂, and PGF_{2α}) in the eicosanoid pathway, which are important in regulation of body temperature, production of hormone and prevention of platelet aggregation (15,16). Non-catalytic binding region on the GSTs allows the enzymes to function as a ligandin that regularly transports endogenous compounds such as bile salts, heme, and bilirubin to avoid excessive accumulation of molecules in the membrane or within the cell (17). These discoveries on the functions of GST marked the importance of the enzymes in body metabolism and human wellness.

GST superfamilies

Glutathione s-transferases (GSTs) isoenzymes are classified into two distinct superfamilies (13). The larger superfamily is composed of cytosolic or soluble enzymes, which have at least 16 genes that encode proteins with GST activity in tissue cytosols and sub classified into eight classes as shown in Figure 1 (2,18). Classification of the GST genes into different classes was based on the degree of sequence identity. Each gene in the same GST class exhibit more than 60% sequence similarity between each other but showing less than 25% similarity with the genes from other GST classes (19). In comparison to other seven classes, *GSTK* is frequently found in mitochondria compared to cytoplasm in soluble state (20). Additionally, *GSTM* has the most complex structure among the classes as there are a cluster of five genes located on chromosome 1 (19). Unlike *GSTM*, other classes such as *GSTZ* and *GSTP* have simpler structure due to only a single gene present in each class (21,22).

Meanwhile, the other superfamily is microsomal GSTs that are categorized as membrane associated proteins in eicosanoid and glutathione metabolism (MAPEG) and comprised of at least six genes (Fig. 1) (23,24). Microsomal GSTs possess diverse functions such as leukot-

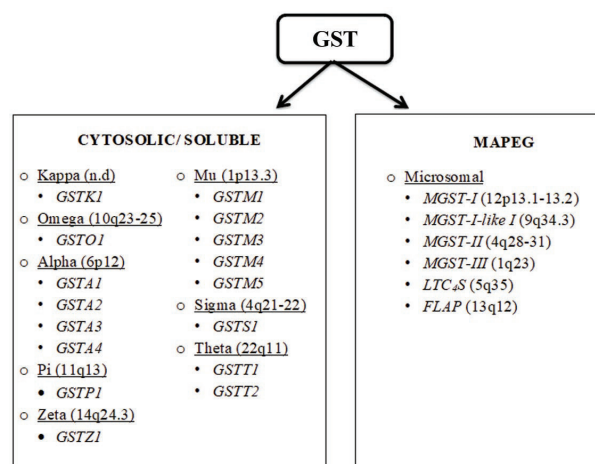


Figure 1: GST classification. GST enzymes are subcategorized under two superfamilies; cytosolic/ soluble GST and MAPEG. Each gene (italic) encoding for respective superfamily enzyme is located on distinct chromosomal location in parentheses.

riene biosynthesis and cytoprotection by exhibiting glutathione s-transferase and peroxidase activities (18,24). Among these two superfamilies, cytosolic GSTs (*GSTA*, *GSTK*, *GSTM*, *GSTT*, and *GSTO*) are the main proteins in detoxifying xenobiotics and endobiotics as they are extensively expressed in the liver (i.e. the major organ for detoxification) (18).

Genetic Polymorphism of GSTs and Their Relevancy

Genetic polymorphism can be referred as the existence of DNA sequence variants in a population at a minimum rate of 1% (25). It arises from any mutations in normal DNA segment that are mainly caused by unrepaired DNA damage and deletion or insertion in certain nucleotide sequences due to mobile genetic elements (26). These events may provoke a point mutation where the mutated variant is different from the normal nucleotide sequence in a single nucleotide base (single nucleotide polymorphisms (SNPs)) as well as alteration of the whole chromosomal structure by translocation, deletion, duplication or inversion of a certain chromosomal region (26).

Mutations in DNA sequences that encoding for important organs and working body systems may impair and modify human physiological system (27). Chances of the mutations to occur are varying among individuals and may lead to inter-individual difference in health and development process (27). In view of detoxification system, variations in the genes encoding GSTs may have the potential to cause alteration and dysfunction of the enzymes that affect the xenobiotic and drug biotransformation process (28). SNPs mutation were most commonly found in *GSTT*, *GSTM*, *GSTA*, and *GSTP* but null allele (i.e. *GSTM1*0*, *GSTT1*0*) which is known to have complete deletion of the gene only involves with *GSTM* and *GSTT* (28). Null allele affects the functions of enzyme more significantly than other allele variants. The

loss of gene accounts for less production of gene products or enzyme as the gene-protein transcription process is blocked and results in a complete loss of enzymatic function (29).

Genetic Polymorphism of *GSTM1*

GSTM has been expressed in liver and identified to have five isoenzyme subunits: *GSTM1*, *GSTM2*, *GSTM3*, *GSTM4* and *GSTM5* as shown in Fig.2 (19,30). Altogether, those genes clustered at chromosome 1p13.3 with the total size of 100-kb and the amino acid sequence similarity of 85% between each gene. Among the *GSTM* subunits, *GSTM1*, *GSTM2*, and *GSTM3* show a high sequence similarity to *GSTM4* with more than 70% (31). However, the catalytic efficiency of each gene was somehow determined to be significantly different. This is due to the evolution of the genes specifically in the GST binding sites to tackle a wide scope of substrates (32). Additionally, *GSTM1* is the most abundant and highly polymorphic cytosolic GST, alongside *GSTT1*, *GSTT2*, *GSTA1*, *GSTA2*, and *GSTP1* in human compared to other *GSTM* subunits, which makes it as the gene of interest in numerous physiological and pharmacological studies (33).

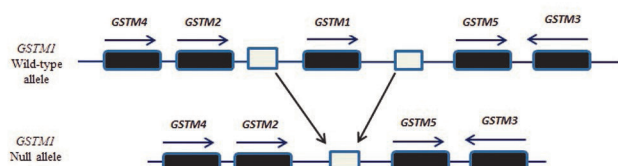


Figure 2: Mechanism of *GSTM1* polymorphism. *GSTM1* is being located on chromosome 1 alongside other five *GSTM* subunits and embedded by two homologous regions (white box) in the normal/wild-type allele. However, the null allele will be produced when homologous recombination between the two flanking regions occurs and *GSTM1* gene is completely excised.

GSTM1 is located in between of *GSTM2* and *GSTM5* and separated from these two genes by the presence of 4.2 kb homologous flanking regions at each side of the gene locus (9) as illustrated in Fig.2. The two flanking regions are probable to recombine and result in the absence of 16-kb *GSTM1* locus. The deletion polymorphism produces a null allele (*GSTM1*0*) and *GSTM2* will be subsequently located next to *GSTM5* (34,35). However, the highly identical repeats between the two regions makes it difficult to determine the deletion junction. In addition, exon 8 of *GSTM1* and *GSTM2* genes have a high sequence similarity of 99% and an unequal crossing over has a high tendency to occur between the two gene loci to produce *GSTM1*0* (19,36). The absence of the *GSTM1* gene results in no expression of the enzymes.

Apart from *GSTM1* null allele (*GSTM1*0*), *GSTM1* has other 3 allelic variants which are *GSTM1*A*, *GSTM1*B* and *GSTM1*1X2*. A single nucleotide substitution at position 534 results in two alleles: *GSTM1*A* (G) and *GSTM1*B* (C) containing different amino acid product at

codon 172, which are lysine and asparagine, respectively (37). However, these two alleles are not functionally different from each other as both did not show any significant contribution to the alteration of enzymatic function in any previous studies (28). Meanwhile, an overexpression of *GSTM1* was presented in *GSTM1*1X2* allele which resulted from gene duplication. Hence, this particular allele elevates the functionality of GST enzymes (12). Other than *GSTM1*, no studies have reported any deletion polymorphisms in other *GSTM* isoenzymes, specifically deletion of the entire gene, and its association with reduced metabolic capacity. In regard to this, only *GSTM1* have been investigated in large-scale studies and signified its significance in toxicology and cancer epidemiology studies.

Genetic Polymorphism of *GSTT1*

Two loci encode for *GSTT* had been identified to be clustered at chromosome 22q11.2 (38,39). The gene loci were denoted as *GSTT1* and *GSTT2* and separated by ~50kb. These two *GSTT* isoenzymes have ~55% homology which is less compared to other GST classes that have a range of 75-95% of sequence identity among the members of the same class (19,40).

There are two 18kb flanking regions of more than 90% sequence identity (HA3 and HA5), situated downstream and upstream of *GSTT1* as illustrated in Fig. 3. The deletion polymorphism of *GSTT1* (*GSTT1*0*) arise due to the homologous recombination between the 100% identical 403bp sequences located in the center of both flanking regions (41). Consequently, *GSTT1* enzymes are unable to be expressed and the enzymatic activity is lost due to the absence of the gene.

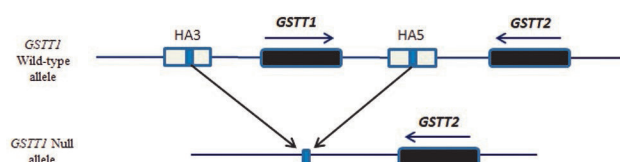


Figure 3: Mechanism of *GSTT1* polymorphism. *GSTT1* and *GSTT2* are arranged on chromosome 22 and separated by two homogenous regions (HA3 and HA5) in the wild-type allele. Homologous recombination is likely to occur between the highly identical sequence of the central portions of HA3 and HA5 which causes the gene locus of *GSTT1* to be deleted in the null allele.

Apart from *GSTT1*0*, *GSTT1* also has other two functional allelic variants which are *GSTT1*A* and *GSTT1*B*, involved in different catalytic activity (42). A single nucleotide polymorphism (SNP) that substitutes amino acid 104 will produce two different alleles, *GSTT1*A* (threonine) and *GSTT1*B* (proline) and modify the enzymatic structure. It alters the level of enzymatic function with *GSTT1*B* showing lower catalytic activity than *GSTT1*A* (42). Unlike null allele, this SNP still results in enzymatic function because the polymorphism only occurs at a single base without interrupting the whole *GSTT1* gene sequence and the enzyme can still be produced (42).

Compared to *GSTT1*, *GSTT2* has only two functional alleles: *GSTT2*A* (methionine) and *GSTT2*B* (isoleucine) that produce different products of amino acid 139. However, the two alleles do not significantly affect the enzymatic activity (42). This shows that *GSTT1* polymorphism is more significant due to its alteration in the enzyme metabolic capacity compared with *GSTT2* polymorphism. Since *GSTT1*0* confers a complete loss of enzymatic activity and down regulates detoxification system, this null allele has been recognized as an important GST marker in toxicological and epidemiological studies (43).

Frequency of *GSTM1* and *GSTT1* null polymorphism

GSTM1 and *GSTT1* have been observed to show ethnic dependent polymorphisms, where it occurs at different frequencies across populations. The frequency of *GSTM1* null is higher in European with 50-57% and followed by African (30-55%), Asian (28-66%) and American (44%) populations. *GSTT1* null genotype is varying from 7%-53% for those of Asian descent; 29%-50% of African descent, 10%-33% of European descent and 12%-18% of American descent (44).

Malaysia is a multiracial country comprising of diverse ethnic groups. Department of Statistic Malaysia reported for 2014-2016, Bumiputera are predominant in Malaysia with 68.6%, followed by Chinese (23.4%), Indians (7.0%) and others (1.0%) of the population (45). Bumiputera consists of Malays and indigenous peoples, including Orang Asli, Dayak, Anak Negeri (45). To the best of our knowledge, there are only few studies reporting on *GSTM1* and *GSTT1* deletion in Malaysia. Nur-fatimah et al., (2011) reported about 59.4% and 32% of Malaysians with deletion of *GSTM1* and *GSTT1*, respectively (46). Different study by Alshagga et al. (2011) revealed the deletion of *GSTM1* occurs at 66% but the frequency of *GSTT1* deletion is lower (18%) (47). However, these studies did not include the other two major ethnic groups, which are the Chinese and Indian. In 2012, Eshkoo et al. had determined *GSTM1* (9.17%) and *GSTT1* (62.5%) deletion in the control population that consisted of the three ethnic groups but did not further differentiate the null polymorphism in each ethnic groups (48). Thus, the relationship between the null polymorphism and ethnicity factor is still undetermined in Malaysia to date. Therefore, few studies have been selected from different populations and continents to highlight and compare the distribution of *GSTM1* and *GSTT1* null polymorphism between Malaysian and other populations (Table I).

The frequency of *GSTM1* deletion in Malaysian population (59.4%) is similar to those reported in Tunisian (54.39%), Sudanese (54.4%) and German (57%) populations but higher than those reported in Iranian (28%), Indian (33.3%), and Nigerian (30%) (5,49–52). Meanwhile, the frequency of *GSTT1* deletion in Malaysian population (32%) is consistent with Indian (24.4%) but

Table I: Frequency of *GSTM1* and *GSTT1* null genotypes in Malaysian and worldwide populations. Null polymorphism of *GSTM1* and *GSTT1* are observed at different frequencies among the distinct populations. The high frequency of *GSTM1* null in Malaysian population is consistent with majority of the populations. Unlike *GSTM1*, the frequency of *GSTT1* null appears to be diverged between Malaysian population and most of the populations.

Population	N	Assay	Null genotype (%)			Reference
			<i>GSTM1</i>	<i>GSTT1</i>	<i>GSTM1/GSTT1</i>	
Malaysian	128	Multiplex-PCR	59.4	32	16.4	(46)
Chinese	412	Duplex PCR	47.1	48.1	21.86	(57)
Iranian	336	Multiplex PCR	28.0	20.8	12.5	(5)
Turkish	231	Multiplex PCR	53.68	18.61	20.40	(56)
Indian	492	Multiplex PCR	33.3	24.4	7.9	(51)
Korean	244	Multiplex PCR	57.4	52.5	30.7	(4)
Tunisian	182	Multiplex PCR	54.39	29.12	18.68	(50)
Nigerian	300	Multiplex PCR	30	37	13	(49)
Sudanese	114	Multiplex PCR	54.4	42.1	24.6	(53)
Moroccan	93	Multiplex PCR	40.9	9.7	5.4	(58)
Caucasian	105	Multiplex PCR	56.2	21	16.2	(55)
Italian	548	Multiplex PCR	50.4	16.7	7.7	(54)
German	93	Real-Time PCR	57	18	10.8	(52)
Brazilian	147	Real-Time PCR	43.5	12.2	4.1	(59)

*N- number of control subjects; PCR-polymerase chain reaction

higher than in Iranian (21%), Turkish (18.61%), Caucasian (21%) and Italian (16.7%) populations (5,51,53–56). However, Chinese and Korean populations have higher prevalence of *GSTT1* null with 48.1% and 52.5%, respectively (50,57). The occurrence of deletion polymorphism in both *GSTM1* and *GSTT1* genes in Malaysia is less common with the frequency at 16.4% only (46). It is relatively higher than the frequency reported in Brazilian (4.1%), Moroccan (5.4%), Italian (7.7%), Indian (7.9%) and German (10.8%) populations but lower than Koreans, which has the highest frequency at 30.7% (51,52,54,58,59).

The distribution patterns of *GSTM1* and *GSTT1* polymorphisms are illustrated in Fig. 4. *GSTM1* gene is observed to be absent in most of the populations at high

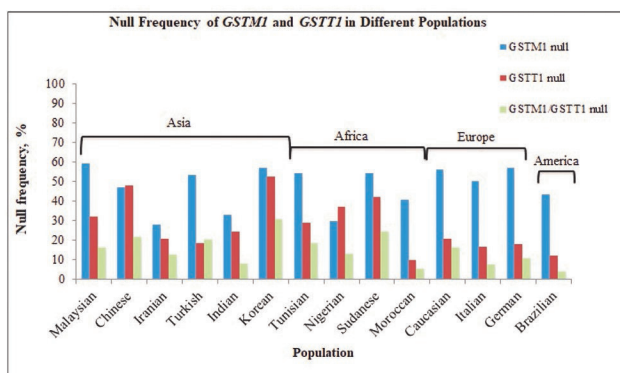


Figure 4: Distribution of *GSTM1* and *GSTT1* null genotype in world-wide populations. Null polymorphism of *GSTM1* and *GSTT1* are observed at different frequencies among the populations in four main continents. All four continents shows high frequencies of *GSTM1* null whereas *GSTT1* are commonly deleted in Asia and Africa only compared in other two continents. The incidence of homozygous deletion of both genes is lower compared to the deletion of either one gene for each population.

frequency. In Asia continent, the deletion mutation in *GSTT1* varies across the different countries, including Malaysia, compared in *GSTM1* that makes the determination of *GSTT1* status in Asia regions is difficult. Apart from the inter-ethnic differences, an evolutionary basis may contribute to the contrariety findings. Genetic drift may present in all populations; however, the effects of the evolutionary mechanism such as random sampling and bottleneck effect are likely to become obvious in some of small Asian populations (60,61), including Malaysia. For example, the probability of the null alleles to be selected and passed down to the next generations in each population might be imbalanced, which is higher or lower in one population compared to other populations. Therefore, the genetic variation is most likely to appear between Asian countries.

Significance of *GSTM1* and *GSTT1*

As the dominant enzymes in detoxification system, gene expression of GSTs is important to resist a wide range of carcinogens and toxicants from drugs or environment. Deletion mutation is considered as minor polymorphism (<10%) that occurs in human population whereas single nucleotide polymorphisms (SNPs) (90%) are more common. However, deletion polymorphism showed to greatly affect carcinogen metabolism compared to SNPs (62). Thus, this explains the homozygous deletion of *GSTM1* and *GSTT1* have been intensively explored in more than 500 publications in molecular epidemiology (9). The deletion of the two genes is predicted to impair the detoxification of carcinogens in the body and the null individuals are exposed to high risk of cancers and diseases such as lung (6) and gastric cancers (57). Thus, investigations on these particular genes can contribute beneficial values to the knowledge in genetics and cancer etiology. The knowledge can lead to the awareness of the danger of environmental pollutants, drugs and dietary agents that presents in our daily life (63). However, there are certain situations where the level of GSTs needs to be down regulated such as for chemo-

therapy purpose (64). High GST activity will reduce the effectiveness of anticancer drugs and build up resistance towards chemotherapy agents (64). The current alternative is to produce a suitable GST inhibitors which can work efficiently to achieve a good therapeutic index of the drugs (64).

Relevance of GST polymorphism to disease susceptibility

As the main character in detoxification of a wide range of carcinogens and toxicants from drugs or environment, *GSTM1* and *GSTT1* deletion could render individuals to be more susceptible to diseases or cancers due to abolished enzymatic activity. Absence of the genes has been linked with several main cancers in a number of studies as shown in Table II.

Nullity of *GSTM1* and *GSTT1* has been commonly found to be associated with increased risk of lung cancer (LC) as null individuals are hypothesized to be unable to detoxify the main carcinogens involved in the carcinogenesis such as benzo[a]pyrene, nitrosamine, and aromatic amines that present in tobacco smoke (6,65,66). Homozygous null genotype (-/-) of each gene was significantly emphasized to be a stronger candidate risk factor of LC through trimodal genotype analysis (+/-, +/-, -/-) in a Caucasian study (65). However, the deleterious effect of GSTs on LC could not be replicated in few studies. Both genes failed to be marked as the genetic factor to the cancer development in Bangladeshi population whereas a South India study showed only *GSTT1* null individuals were susceptible to the cancer in the population (67,68). These contradicting findings might indicate the impact of ethnicity. However, small sample size and less detailed information about other risk factors (i.e. residential area and smoking status) in some of the studies, might also explain for the heterogeneity. In contrast, *GSTT1* polymorphism showed a protective effect against the development of LC in North East Indian population (10). Gene-gene interaction is plausible to cause the null effect being masked by functional compensation of *GSTT1* null with other GST or metabolic genes. Despite its role in detoxification, *GSTT1* also involves in activation process of certain toxic compound which is advantageous for null individuals (69).

Reduction of GSTs' enzymatic activity is also responsible to high risk of gastric cancer (GC) among individuals with null genotype. *GSTM1* and *GSTT1* null genotypes were considered as the potential risk modifiers for GC among the exposed group of Italians and Chinese (70,71). However, such associations were undetermined in other case-control studies. Although a Korean study involved greater sample size and a similar study was conducted in Chinese population, both failed to prove the modification effect of GST polymorphisms on GC risk including in Spanish population (72-74). Other than ethnicity factor, selection of respondents in both cases and controls group might be the reason for

Table II: Association between *GSTM1* and *GSTT1* polymorphism and disease susceptibility worldwide. Null polymorphism of *GSTM1* and *GSTT1* are observed to significantly modified the risk of cancers or diseases in some of the populations. Meanwhile, inverse associations were observed in few studies or populations.

Diseases	Year	Population	N		Matching Criteria	Sample	Genotyping Assay	Null genotype		Reference
			Case	Control				<i>GSTM1</i>	<i>GSTT1</i>	
Lung cancer	2012	Caucasian	2100	2120	Age, sex, and residential areas	Blood	Duplex TaqManR Real-Time PCR	↑	↑	(66)
		Chinese	110	100	Age, sex, smoking status	Blood	Multiplex PCR	↑	↑	(67)
	2014	North East-Indian	154	154	Age, sex, smoking status	Blood	Real-Time PCR	-	↓	(10)
		Bangladeshi	106	117	Age, sex, smoking status	Blood	Duplex PCR	-	-	(69)
	2015	North Indian	270	270	Age, sex, smoking status	Blood	Multiplex-PCR	↑	↑	(6)
	2016	South Indian	246	250	Age, sex, smoking	Blood	Multiplex PCR	-	↑	(68)
Gastric cancer	2009	Korean	2213	1699	Age, sex, drinking, smoking status	Blood	Real-Time PCR	-	-	(73)
	2010	Italian	314	548	Age, sex, <i>H.pylori</i> serology, family cancer history, residential area, smoking	Blood	Multiplex PCR	↑	↑	(72)
	2012	Spanish	557	557	Age, sex, and residence areas	Blood	Multiplex PCR	-	-	(74)
	2012	Chinese	410	410	Age, sex, <i>H.pylori</i> infection, smoking	Blood	PCR-CTPP	↑	↑	(71)
	2017	Chinese	242	396	Age, sex, drinking, smoking, family cancer history and intake of pickled food.	Blood	Multiplex PCR	-	-	(75)
	2007	Turkish	98	98	NA	Blood	Real-Time PCR	↑	-	(76)
Diabetes Mellitus	2013	Brazilian	120	147	Age, sex, smoking, and drinking	Blood	Multiplex SYBR Green Real-Time PCR	-	↑	(59)
	2016	Malaysian	151	136	Age, sex, ethnic	Blood	Multiplex PCR	-	-	(77)
	2009	Korean	1829	1699	Age, sex, drinking, smoking status	Blood	Real-Time PCR	-	-	(73)
Colorectal cancer	2011	Malaysian	111	128	NA	Blood	Multiplex PCR	-	↑	(46)
	2014	Chinese	264	317	Age and sex	Blood	Multiplex PCR	↑	↑	(78)
	2018	Polish	197	104	NA	Blood	qPCR relative quantification method	↑	↑	(8)
Bladder cancer	2013	Turkish	114	114	Age, sex	FFPE	Multiplex PCR	-	↑	(80)
	2016	Pakistani	236	270	Age, sex, residential areas, smoking status	Blood	Multiplex PCR	↑	↑	(79)
	2018	Algerian	175	188	Age, sex, and ethnicity	Blood	Multiplex PCR	↑	↑	(7)
Prostate cancer	2011	Iranian	168	336	Age and smoking status	Blood	Multiplex PCR	↑	↑	(5)

*N-number of volunteers; NA- Not available in original article; FFPE: Formalin fixed paraffin embedded (nontumoral bladder tissues); ↑ - Increased risk of the specific cancer; ↓ - Reduced risk of the specific cancer; '-' - No association observed between the null polymorphisms and cancer

the discrepancies. Most of the studies recruited hospital-based controls which produced high probability to involve individuals having defective genes due to other diseases. Moreover, risk of GC was heightened in the simultaneous analysis of GST polymorphism and other contributing factors: *Helicobacter pylori* (*H.pylori*) infection, smoking, and alcohol drinking. Thus, lacking of the variables in the matching criteria produced biased results and unable to utilize conditional logistic regression models efficiently.

A strong association between the occurrence of diabetes mellitus type 2 (T2DM) and *GSTM1* and *GSTT1* polymorphism has also been observed as the oxidative stress products produced from the disease pathogenesis are unable to be detoxified by the enzymes (58,75). The modifying effect of *GSTM1* null was reported in Turkish patients whereas, Brazilian patients were only affected by the absence of *GSTT1* gene (58,75). Meanwhile, both genetic polymorphisms showed no association with risk of T2DM among Malaysians (76). With similar sample size and assay, conflicting findings between *GSTM1* and *GSTT1* null and T2DM pathogenesis could be attributable by the ethnicity factor. Likelihood of variation at the two gene loci in each ethnic's ancestry line and predisposition to T2DM are different among ethnics.

Null variant in *GSTM1* and *GSTT1* has also been demonstrated to confer colorectal (CC), bladder (BC), and prostate cancer (PC) susceptibility in different populations (5,7,8,77,78). However, some of the findings diverged considerably with other studies, causing such associations remain inconclusive to date. Even though the null genotypes were analysed in a large group of cases and controls, a Korean study revealed no strong relationship between the genetic polymorphism and CC (72). Meanwhile, only *GSTT1* null signified elevated risk for CC susceptibility among Malaysian patients (46). In addition, *GSTM1* null showed to have no impact for risk of BC in Turkish population compared to Pakistani and Algerian (79). These disparities for both cancer cases may be due to the inappropriate logistic regression used for matched case-control study, which was unconditional logistic regression analysis. This analysis had shown weak precision of evaluation and hypothesis of the study when compared to conditional logistic regression analysis (80) (Kuo et al., 2018).

To date, case-control studies on GST-associated diseases in Malaysia is consider still relatively less investigate to the influence of GST polymorphism on those common cancers in the population. As Malaysia is a multiethnic country, it is relevant to conduct epidemiological studies to further signify the impact of ethnicity on the risk of disease incidence. Considering all the factors mentioned above, this review calls for future epidemiological or case-control studies in Malaysian and other populations to be well designed with appropriate study design, measurements or variables, sample size, geno-

typing assays, and statistical analysis to precisely determine the relevance of GST polymorphism on disease susceptibility. Thus, the need to analyse data combining from numerous studies to reach general consensus on the associations can be reduced.

Genotyping assay for *GSTM1* and *GSTT1*

Conventional PCR is widely employed in numerous fields such as forensic, medical genetics, species identification, genetic mutations and polymorphisms by replicating a target fragment in a trace amount of DNA into billion copies (81). In the early years of GST genotyping, restricted fragment length polymorphism (RFLP)-PCR technique has been performed to genotype the possible allelic variants of *GSTM1* (*GSTM1**A, *GSTM1**B and *GSTM1**O) simultaneously (69). However, this approach become less popular as both *GSTM1**A and *GSTM1**B alleles showed insignificant compared to the null allele (*GSTM1**O), which was found to affect the carcinogen metabolism significantly.

To reveal more than one genetic variant in a DNA sample robustly, multiplex PCR has been developed to amplify multiple target genes of interest in a single reaction, otherwise it would require several reactions preparation, use more reagents and time in single PCR (82). Over the years, this method has been applied in a large-scale of *GSTM1* and *GSTT1* studies and co-amplified with an internal control that known to be constitutively present in humans such as β -globin and Albumin (ALB) genes (3,83,84).

Besides, real-time PCR is also a common tool used to genotype the polymorphism of *GSTM1* and *GSTT1* genes. This assay offers detection on copy number changes of both *GSTM1* or *GSTT1* alleles and distinguish homozygous wild type (+/+) or null (-/-), and heterozygous null (+/-) genotypes. For example, a case control study by Zhou et al. (2010) has successfully extrapolated the impact of heterozygosity of *GSTM1* or *GSTT1* on age-related cataract (ARC) by differentiating the carriers of one (+/-) and two (-/-) null alleles using the quantitative real-time PCR (85). On the other perspective, the information on heterozygosity status of the genes will be valuable in the view of Hardy-Weinberg equilibrium (HWE). The presence of genetic polymorphism could be use to investigate the evolutionary forces over the generations in a population. Contrary, conventional PCR exclusively determines the homozygous gene deletion in the absence of gene products but unable to discriminate the heterozygous (+/-) from wild type (+/+) genotype (86). However, genotyping of heterozygous deletion for both *GSTM1* and *GSTT1* is less significant because the effect of the genes appeared to be significant in homozygous null individuals (87).

CONCLUSION

Modification on both *GSTM1* and *GSTT1* expression

could influence the biotransformation or detoxification of carcinogens. Different distribution of *GSTM1* and *GSTT1* polymorphisms are found between ethnic groups and across the population gives an insight of the human genetic diversity. Regards to the polymorphism consequences on the health status, GST could be serve as a marker to explain and predict inter-individual differences in cancer risks. It also contributes a valuable knowledge to medicine field in improving diagnostic and therapeutic strategies for the cancers. However, the epemiological studies should be executed using a well-designed of study by taking consideration on gene-gene interaction, larger sample size, selection bias of case and control groups, other relevant contributing factors and appropriate genotyping assay to robustly extrapolate the associations between GST polymorphisms and multifactorial cancer susceptibility with an adequate statistical power. Conventional PCR-based assay is still a simple, inexpensive and common method to genotype the null variant solely to observe the deletion effects. Yet other approaches either different assays or modified-PCR are necessary to provide more meaningful information on HWE and detailing out the effect of gene-dosage on disease risks. As a multi-ethnics country, comprehensive studies should be conducted to determine the genetic differences related to cancer susceptibility on the basis of ethnicity in Malaysia and may lead to the personalized medicine.

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